PATENT
Atty. Docket No. 2303.2B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	David H. Gelfand et al.	
SERIAL NO.	:	07/387,003	GROUP ART UNIT: 187
FILED	:	July 28, 1989	EXAMINER: E. Steffe
DOCKET NO.	:	2303.2B	
TITLE	:	PURIFIED THERMOSTABLE ENZYME	

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.56, §1.97 AND §1.98

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

The citations listed on the enclosed P.T.O. 1449 form and the information provided in this statement may be material to the examination of the above-identified patent application and are submitted in compliance with the duty of disclosure as defined in 37 CFR §1.56, §1.97, and §1.98. The Examiner is requested to make this information of official record in the application.

The present invention provides stabilized thermostable nucleic acid polymerase enzyme compositions. The present inventors are the first to recognize that to prepare purified thermostable nucleic acid polymerase of commercial quality, one must stabilize the purified preparation with non-ionic detergents. The resulting compositions made by the inventors have met with widespread commercial success.

Prior to 1985, the relatively rudimentary work performed on the purification of thermostable nucleic acid polymerases had not resulted in any widespread use of such enzymes. However, in 1985, Dr. Kary Mullis of Cetus Corporation invented a nucleic acid amplification technique now widely known as the polymerase chain reaction (PCR).

This technique involved target dependent replication of nucleic acids by a cyclical process in which oligonucleotide primers were extended on the template strands of the target. Because the primer extension products serve as templates for synthesis of primer extension products in later cycles, the target sequence accumulates exponentially in relation to the number of cycles of primer extension.

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By [signature]

The primer extension reaction of PCR is typically performed with a nucleic acid polymerase enzyme and with a heat denaturation step at the end of each cycle of primer extension. This latter step irreversibly inactivates non-thermostable polymerase enzymes, and the inventor of PCR recognized that thermostable polymerase-mediated PCR was an efficient embodiment of his invention.

The present inventors worked at Cetus with Dr. Mullis and began to study thermostable DNA polymerases after Dr. Mullis invented PCR. The scientific publications available to the present inventors did not, however, provide sufficient guidance to allow one of ordinary skill to purify a thermostable polymerase of commercial quality.

The inventors' initial efforts were directed at purifying the thermostable polymerase of Thermus aquaticus. The inventors discovered that the prior art failed to teach the most useful form of DNA polymerase from T. aquaticus. Instead, the art described crude preparations that at most contained degraded forms of another far more useful enzyme. The successful purification of T. aquaticus DNA polymerase I (Taq polymerase) was first described in now abandoned U.S. patent application Serial No. 899,241, filed August 22, 1986.

The inventors quickly discovered improved methods of purification and recombinant DNA cloning vectors for producing purified Taq polymerase. In these efforts, the inventors realized that thermostable nucleic acid polymerase enzymes in general, and Taq polymerase enzyme in particular, has properties unlike the heat-labile nucleic acid polymerases of the prior art.

One such property relates to the stability of the enzyme throughout the purification of the enzyme and during storage of purified preparations. The inventors discovered that the presence of non-ionic detergent in the purified preparations significantly improved stability of the enzyme.

The inventors described these embodiments of their invention in continuation-in-part (of the '241 application) application, Serial No. 063,509, filed June 17, 1987. This application was prosecuted to issue as U.S. Patent No. 4,889,818. The claims of the '818 patent focused on purified preparations of Taq polymerase.

A CIP of the '509 application, co-pending Serial No. 143,441, was filed on January 12, 1988, prior to issuance of the '818 patent. This CIP application subsequently served as the parent of a number of divisional applications: the present and Serial No. 381,174, both filed on July 28, 1989. The '441 application supports claims to recombinant DNA vectors and compounds encoding thermostable nucleic acid polymerases.

The '174 application is drawn to a genus invention: purified, thermostable nucleic acid polymerases, of which Taq polymerase is a species. The present application is drawn to another genus invention, and a number of species of that genus. The stabilized enzyme compositions of the present invention have been recognized as a patentably distinct invention from the invention claimed in the '818 patent (and the pending '174 divisional application and '441 parent application) by the Patent and Trademark Office.

This fact is apparent from the file wrapper of the parent '241, '509, and '441 applications. Consequently, Applicants respectfully request that Examiner review the file wrappers of the related applications discussed above, with particular attention to the disclosure statements and Office Actions of record, in considering the patentability of the present application.

Examiner should also be aware of two commonly owned, co-pending applications relating to purified thermostable nucleic acid polymerases. Serial No. 455,611, filed December 22, 1989, describes methods for the reverse transcription of RNA with a thermostable DNA polymerase. Serial No. 455,967, filed December 22, 1989, describes methods for purifying and expressing in recombinant host cells Thermus thermophilus DNA polymerase I.

To aid Examiner, Applicants provide copies of the following references and discussion. To facilitate that discussion, the references are summarized in tabular form below.

U.S. Patent No.	Published European Patent Applications	Published PCT Patent Applications
4,683,195		
4,683,202	258,017	89/06691
4,889,818		

The '195 and '202 patents describe the PCR nucleic acid amplification process and various applications of that process, including thermostable polymerase-mediated PCR processes. These latter processes are more fully described in copending Serial No. 063,647, filed June 17, 1987.

The relevance of the '818 patent has been discussed above, and Applicants reiterate that a number of related applications are pending before the P.T.O. European patent publication No. 258,017 is the foreign counterpart of Serial Nos. 063,647 and 063,509, and published March 2, 1988, after the filing date (January 12, 1988) of the parent (Serial No. 143,441) of the present Rule 60 divisional application. Applicants have also enclosed a copy of the Search Report

for EP 258,017. If Examiner would like to review a copy of any reference listed on the Search Report and not submitted with the present statements, then Examiner need only request Applicants to provide a copy.

The '441 application was filed in the PCT and published on July 27, 1989, as publication No. 89/06691. Applicants have provided a copy of the publication and both Search Reports for the PCT application.

Scientific Journal Publications

Stenesh and Roe, 1972, Biochim. Biophys. Acta. 272:156-166, describes efforts to purify a DNA polymerase from two Bacillus species: B. licheniformis and B. stearothermophilus. Examiner should consider the passage at page 161 of the reference, where the authors note: "This fraction was stored at -20°C and lost about 50% of its activity within 1 month."

Air and Harris, 1974, FEBS Letters 38(3):277-281, describe efforts to purify a DNA-dependent RNA polymerase from Thermus aquaticus. The article does not describe stabilized compositions of the present invention.

Edgar et al., 1975, Abst. ASM Ann. Mtg. 75:151 (Abst. No. K26), and Chien et al., 1976, Chem. Abst. 85:180 (Abst. No. 155559t), originate from the laboratory of Dr. John Trela and describe efforts to purify a DNA polymerase from Thermus aquaticus. The abstracts are believed to be cumulative with the J. Bact. article discussed below. It is interesting to note that one abstract states the molecular weight of the enzyme as 72 kD, but the other abstract and article reports a molecular weight of 63-68 kD.

Chien et al., 1976, J. Bact. 127(3):1550-1557, describe these efforts in greater detail. Examiner should note that in Figure 1, several faint bands appear in the tube gel labelled B, to the left of the heavy BSA bands in the tube gel labelled A. One of the fainter bands in tube gel B aligns with the peak activity in the graph immediately below the gels. These faint bands are often wholly absent in copies of the original article. At page 1555, the authors report that a "stable thermophilic DNA polymerase has been isolated" (emphasis added). The reference does not describe the stabilized enzyme compositions of the present invention.

Fabry et al., 1976, Biochim. Biophys. Acta. 435:228-235, describes efforts to purify a thermostable RNA polymerase from Thermus aquaticus. At page 229, the authors describe their storage buffer and report storage stability at -20°C for several months. The reference does not describe the stabilized enzyme compositions of the present invention.

Kaledin et al., 1980, Chem. Abst. 93:377 (Abst. No. 40169p), is cumulative with Kaledin et al., 1980, Biochem. 45(4):494-501. The references describe efforts to isolate a thermostable DNA polymerase from Thermus aquaticus. The authors state, at page 497 of the Biochem. article, the following information about the stability of their polymerase preparations:

> The introduction of gelatin stabilizes the enzymatic activity. If gelatin is not introduced in this stage, the enzyme quickly loses its activity. DNA polymerase is also stabilized by albumin, but when it is used, it is difficult to determine the specific activity at 70°C.

The authors' efforts to purify the enzyme were beset by stability problems, problems solved for the first time by the present inventors.

Kaboev et al., 1981, J. Bact. 145(1):21-26, describe efforts to purify DNA polymerase I from Bacillus stearothermophilus. The authors report, at page 25, "preservation of high activity during long storage," but do not describe the stabilized compositions of the present invention.

Kaledin et al., 1981, Biochem. 46:1247-1254, describe efforts to purify a DNA polymerase from Thermus flavus. At page 1250, the authors report storage stability at -20°C "for at least three years." The authors do not describe the stabilized compositions of the present invention.

Kaledin et al., 1982, Biochem. 47:1515-1521, and Kaledin et al., 1983, Chem. Abst. 98:298 (Abst. No. 49311q), describe efforts to purify a DNA polymerase from Thermus ruber. At page 1518, the authors state:

> At the last two stages of purification, the necessity arose for stabilization of DNA polymerase Tru in view of its extreme lability on account of the low content of protein in the enzyme preparation. Autoclaved gelatin proved the most effective stabilizer.

The present invention provides a patentably distinct and better solution to the instability problem associated with purified thermostable nucleic acid polymerase compositions.

Ruttiman et al., 1985, Eur. Biochem. 149:41-46, describe efforts to purify DNA polymerases from Thermus thermophilus HB8. The reference does not describe the stabilized compositions of the present invention.

Klimczak et al., 1986, Biochem. 25(17):4850-4855, describe efforts to purify a DNA polymerase from Methanobacterium thermoautotrophicum. At page 4853, the authors report that "after 10 minute pretreatment at 100°C no activity was detected even at the optimal temperature." The reference does not describe the stabilized compositions of the present invention.

Rossi et al., 1987, System. Appl. Microbiol. 7:337-341, describe efforts to purify a thermostable DNA polymerase from Sulfolobus solfataricus. At page 338, the authors state that the "enzyme was stored at 4°C for weeks in 10% or at -20°C in 50% glycerol for long periods without appreciable loss of activity." The reference does not describe the stabilized compositions of the present invention.

Kogan et al., 1987, N. Eng. J. Med. 317:985-990, describe the use of Taq DNA polymerase in PCR. At page 986, the authors report that New England Biolabs supplied the Taq polymerase. This reference does not report whether the Taq polymerase contained stabilizers, but does not report multiple additions of the polymerase during PCR (see page 986), indicating a possible stability problem.

Verhoeven et al., 1988, Abst. ASM Ann. Mtg. 88:214 (Abst. No. K47), describe further efforts in the Trela laboratory to purify a DNA polymerase from Thermus aquaticus. The reference does not describe the stabilized compositions of the present invention.

Saiki et al., 1988, Science 239:487-491, and Lawyer et al., 1989, J. Biol. Chem. 264(11):6427-6437, published after the filing date (January 12, 1988) of the parent (Serial No. 143,441) of the present Rule 60 divisional application. However, these references describe much of the work disclosed in the present application and so may be of interest to Examiner.

In addition to the publications discussed above, Examiner should consider the commercial activities discussed below in deciding on the patentability of the present invention. This discussion is separated into sections headed by a named company.

Molecular Biology Resources (MBR):

Much of the disclosure regarding stabilized enzyme compositions in the present application was originally filed in priority application Serial No. 063,509 on June 17, 1987. Priority application Serial No. 899,241 was filed on August 22, 1986, and after that date, Cetus and MBR entered into a confidentiality and materials transfer agreement. Under this agreement, Cetus provided confidential information, including information regarding the need for and identity of the stabilizers in the present compositions, for MBR to use in preparing purified Taq polymerase for Cetus.

On June 8, 1987, MBR violated this agreement by circulating a product information sheet that described one embodiment of a stabilized thermostable nucleic acid polymerase composition of the present invention. Because the MBR information was derived from the present inventors and

appeared in public only a few days before Serial No. 063,509 was filed, this commercial activity of MBR has no effect on the patentability of the present invention.

Applicants have provided a number of letters exchanged between MBR and Cetus regarding this activity for Examiner's consideration.

New England Biolabs (NEB):

Applicants believe NEB began promoting the release of Taq polymerase sometime in April 1987. However, in October, 1987, NEB released a catalogue update that indicated Taq polymerase was still not yet available. Note from the Kogan et al., article, above, however, that NEB apparently provided Taq polymerase to a few researchers prior to October 15, 1987. Examiner should note that the update mentions that two additions of Taq polymerase might still be required in PCR, which leads the Applicants to conclude that NEB had failed to discover the stabilizers of the present compositions.

Cetus:

In July to October 1987, prior to commercial introduction of Taq polymerase by Perkin Elmer Cetus Instruments (PECI), Cetus provided non-Cetus scientists with free samples of Taq polymerase.

PECI:

Applicants believe PECI first offered stabilized compositions of Taq polymerase for sale in October 1987. An early product description is enclosed.

Currently, a large number of companies are marketing stabilized thermostable nucleic acid polymerase compositions within the scope of the present claims. The widespread commercial success of the present invention demonstrates the non-obviousness of the claimed invention.

Applicants respectfully request Examiner to consider carefully the information provided herein and the accompanying response to Office Action. Applicants believe these papers place this application in condition for allowance and so request Examiner to act favorably on the application.

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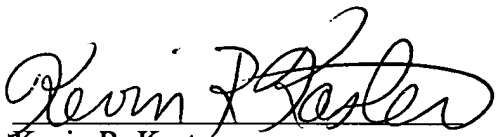
This information disclosure statement under 37 CFR §1.56, §1.97 and §1.98 is not to be construed as a representation that a search has been made, that additional information material to the examination of this application does not exist, or that this citation constitutes prior art under 35 USC §102 or §103.

Respectfully submitted,

By: 

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